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Multi-test analysis and model-based estimation of the prevalence of *Taenia saginata* cysticercus infection in naturally infected dairy cows in the absence of a 'gold standard' reference test

R.M. Eichenberger [a], F. Lewis [b], S. Gabriël [c], P. Dorny [c], P.R. Torgerson [b], P. Deplazes [a,*]

[a] Institute of Parasitology, Vetsuisse-Faculty, University of Zurich, Winterthurerstrasse 266a, CH-8057 Zürich, Switzerland
[b] Section of Epidemiology, Vetsuisse-Faculty, University of Zurich, Winterthurerstrasse 270, CH-8057 Zürich, Switzerland
[c] Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium

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ABSTRACT

The diagnostic values of seven serological tests (ELISAs) and of the obligatory European Union-approved routine visual meat inspection for the detection of *Taenia saginata* cysticercosis were investigated. A total of 793 slaughtered dairy cows were selected in three European Union approved abattoirs in Switzerland, an endemic area (apparent prevalence by enhanced meat inspection up to 4.5%) with typically low parasite burdens. ELISAs based on a somatic larval antigen, isoelectric focused somatic larval antigen, larval excretory/secretory antigens, peptide HP6-2, peptide *Ts*45S-10, pooled peptide solution and a monoclonal antibody antigen capture assay were initially screened. As there is no perfect diagnostic 'gold standard' reference test, the obligatory meat inspection and four selected serological tests were further analysed using Bayesian inference to estimate the "true" prevalence and the diagnostic test sensitivities and specificities. The ELISA for specific antibody detection based on excretory/secretory antigens showed highest sensitivity and specificity with 81.6% (95% credible interval: 70–92) and 96.3% (95% credible interval: 94–99), respectively. The Bayesian model estimated the specificity of the ELISA, based on the synthetic peptide *Ts*45S-10 as 55.2% (95% credible interval: 46–65) and sensitivity as 84.7% (95% credible interval: 82–88). The sensitivity of the ELISA based on mAbs, detecting circulating antigen, was 14.3% (95% credible interval: 9–23) with a specificity of 93.7% (95% credible interval: 92–96). The diagnostic sensitivity of the obligatory standard European Union meat inspection procedure for the detection of *T. saginata* cysticercus infection at the abattoir was estimated to be 15.6% (95% credible interval: 10–23). Based on these data, the modelled prevalence of cysticercosis in dairy cows presented at abattoirs in Switzerland was estimated to be 16.5% (95% credible interval: 13–21). These cattle also had a high prevalence of infection with *Dicrocoelium dendriticum* (60.8%) and *Fasciola hepatica* (13.5%).

1. Introduction

Bovine cysticercosis is caused by the larval infection of the zoonotic tapeworm *Taenia saginata*. Humans are the only definitive host and are infected by consumption of cysts from poorly cooked beef. *Taenia saginata* cysticercosis and taeniosis have a worldwide distribution (Murrell, 2005). Based on abattoir reports, prevalences in cattle of between 0.007% and 6.8% have been reported for different countries in Europe (Anonymous, 2005; Dorny and Praet, 2007).

To prevent human infection, the obligatory standard European Union (EU) meat inspection procedure (EC directive N° 854/2004) represents the only control measure in many European countries. Common routine meat inspection is based on palpation and diagnostic incisions of defined muscles, followed by the search for parasitic lesions localised on superficial and cut surfaces of the inspected carcass (Murrell, 2005). The sensitivity of the current routine meat inspection procedure has been estimated at between 10% and 30% (Dewhirst et al., 1967; McCool, 1979; Geerts et al., 1980; Walther and Koske, 1980; Hayunga et al., 1991; Dorny et al., 2000; Murrell, 2005; Eichenberger et al., 2011). The current practice has a substantial economic impact on agriculture through downgrading the value or condemnation of infected carcasses.

Based on the observation of pronounced antibody mediated immunity following taeniid infection, various ELISAs have been developed for the sero-diagnosis of bovine cysticercosis, based on somatic "crude" worm and larval antigens (Craig and Rickard, 1980; Kyvsgaard et al., 1991), homologous and heterologous antigen preparations (Geerts et al., 1981; Harrison and Sewell, 1981a; Smith et al., 1990), excretory/secretory (ES) products from bovine *T. saginata* or murine *Taenia crassiceps* metacestodes (Harrison and

* Corresponding author. Tel.: +41 (0) 44 635 85 02.
E-mail address: deplazesp@access.uzh.ch (P. Deplazes).

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Sewell, 1981b; Joshua et al., 1988; Ogunremi and Benjamin, 2010), and synthetic or recombinant peptides from potentially protective immuno-dominant determinants (Harrison et al., 1989; Ferrer et al., 2003; Abuseir et al., 2007). Furthermore, assays for the detection of circulating parasite antigens using monoclonal antibodies (mAbs) against ES products of metacestodes are also available (Harrison et al., 1989; Brandt et al., 1992; Van Kerckhoven et al., 1998).

Sero-epidemiological studies have suggested prevalences of 1.11%, 3.09% and 8.83% for north-eastern Spain, Belgium and Lower Saxony in Germany, respectively (Dorny et al., 2000; Abuseir et al., 2010; Allepuz et al., 2012). Sensitivity and specificity are population-specific parameters that vary not only between natural and experimentally infected animals but between different naturally infected populations. To overcome this limitation in diagnostic test evaluation, multiple tests can be used in parallel on a group of animals and an estimate of prevalence and test characteristics can be made. Such an approach within a Bayesian statistical framework has been applied for test evaluation in porcine *Taenia solium* cysticercosis (Dorny et al., 2004).

The present study was designed to compare and evaluate the diagnostic test characteristics of available ELISAs for the serological diagnosis of *T. saginata* cysticercosis in slaughtered cows. A Bayesian approach was used to estimate the prevalence in cows presented at abattoirs in Switzerland and to estimate the sensitivity of the obligatory standard EU meat inspection procedure for the detection of *T. saginata* cysticercus infection.

2. Materials and methods

2.1. Animals and infection status

Sera were collected from three groups (1–3) of cattle. Serum samples were stored at −20 °C until they were tested.

For Group 1, 61 serum samples were collected from dairy cows kept on three average Swiss farms without any history of *T. saginata* cysticercosis. Farm history was followed up to confirm that cysticercosis had never been observed in any slaughtered cattle from these farms for at least 10 years. These presumed infection-free animals provided material to determine the negative/positive threshold (cut-off) for each antibody ELISA.

For Group 2, 793 serum samples were collected from dairy cows at Swiss abattoirs. Of these, 53 samples were collected from carcasses positive for *T. saginata* cysticercosis confirmed during standard or further enhanced meat inspection (Eichenberger et al., 2011). The further enhanced meat inspection protocol incorporated additional morphological or molecular analysis of lesions using PCR primer Cest3/5 according to Trachsel et al. (2007). Of this group, 775 samples were randomly selected during routine meat inspection. A further 18 confirmed positive samples were added for evaluation of the preliminary test performance (Table 1). These 18 samples were not included in the Bayesian analysis as they would have introduced sampling bias. To obtain data regarding liver fluke infection, bile was taken from gall bladders from 449 randomly selected and inspected carcasses from Group 2 animals. Bile samples were examined for eggs of liver flukes using a washing and sedimentation technique (Rapsch et al., 2006).

Sera from nine experimentally infected animals previously used by Abuseir et al. (2007), kindly provided by Dr. Ch. Epe, Institute for Parasitology, University of Veterinary Medicine Hannover, Germany were used for Group 3.

2.2. ELISA for the detection of specific antibodies

All tests were performed in polystrene 96-well microtiter plates (Nunc Maxisorb, ThermoScientific, Germany). Optimal performances for antibody ELISAs were determined by preliminary titration with regard to dilutions of test-specific antigens, sera and secondary antibodies, respectively. Positive control sera from animals with proven infections and negative control sera from farms without any history of *T. saginata* cysticercus infection were included in all tests to adjust for day-to-day and for plate-to-plate variations. Quantification of the protein concentrations of the antigens used was calculated by the Bradford protein assay using the Bio-Rad Protein assay (Bio-Rad Laboratories, USA).

2.2.1. Somatic "crude" T. saginata metacestode antigen (TsmAg)

Viable cysticerci were dissected from the muscles of naturally infected cows after being detected during routine meat inspection. The cysticerci were thoroughly washed in sterile PBS. Crude extracts of somatic antigen were prepared according to Deplazes et al. (1990) and purified by affinity chromatography based on recombinant protein G sepharose (Protein G Sepharose 4 Fast Flow, GE Healthcare, UK) according to the manufacturer's instructions. Flow from the column containing the antigen was stored at −80 °C until used. The ELISA was performed according to Staebler et al. (2006). Optimal test performance occurred with 10 μg/ml of antigen preparation in coating buffer (0.1 M carbonate/bicarbonate buffer, pH 9.6), a serum dilution of 1:200 in blocking buffer (PBS pH 7.2 containing 0.02% NaN_3, 0.05% bovine haemoglobin (Fluka, USA) and 0.2% (v/v) Tween-20) and alkaline phosphate labelled goat anti-bovine IgG (H + L) antibody (KPL, USA) at a dilution of 1:1000 in blocking buffer.

2.2.2. Protein fraction of TsmAg purified using isoelectric focusing (TsmIEF)

A crude extract of *Ts*mAg was fractionated by isoelectric focusing using the Bio-Rad Rotofor® System for fractionating complex protein samples according to the manufacturer's instructions (Rotofor® Preparative IEF Cell, Bio-Rad Laboratories, USA). The protein fraction with an isoelectric point in the pH-range 4.5–5.0 was further used, since this fraction did not cross-react with sera from cattle infected with *Fasciola hepatica*. Antigen was stored at −80 °C until used. The ELISA was performed as described in Section 2.2.1 for *Ts*mAg. However, in the *Ts*mIEF ELISA, the antigen was applied at a dilution of 1:20 in coating buffer. This optimal dilution was determined by titration in the absence of any possible quantification of the protein concentration.

2.2.3. ES antigens of T. saginata metacestodes (TsmES)

Viable cysticerci were dissected from muscle tissue of naturally infected animals and cultured in vitro according to Ogunremi and Benjamin (2010). Groups of seven cysticerci were cultured in 10 ml of medium at 37 °C and 5% CO2 for 20 days. Culture medium containing the antigen was harvested weekly and centrifuged (2,000*g* for 10 min.). Supernatant was stored at −80 °C until used. ELISA was performed with ES antigens (10 μg/ml) in coating buffer corresponding to Ogunremi and Benjamin (2010). Sample dilutions and secondary antibody conditions were used as described in Section 2.2.1 for *Ts*mAg.

2.2.4. Peptide antigens

Commercial synthesised peptides HP6-2 and *Ts*45S-10 (Ferrer et al., 2003) were included in the study. Peptides had a purity of at least 80% confirmed by the manufacturer (ANAWA Trading SA, Switzerland) using high-performance liquid chromatography and mass spectrometry. Peptide-ELISAs were performed using peptide HP6-2 or *Ts*45S-10 or both peptides pooled (1:1), diluted in coating buffer according to Abuseir et al. (2007). The ELISA procedure was performed as described in Section 2.2.1 for *Ts*mAg, except the blocking buffer contained 1% ovalbumin (Sigma–Aldrich, USA) instead of bovine haemoglobin. Serum samples were diluted 1:100 in the modified blocking buffer.